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A novel model of combined neuropathic and inflammatory pain displaying long-lasting allodynia and spontaneous pain-like behaviour

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ABSTRACT

Many clinical cases of chronic pain exhibit both neuropathic and inflammatory components. In contrast, most animal models of chronic pain focus on one type of injury alone. Here we present a novel combined model of both neuropathic and inflammatory pain and characterise its distinctive properties. This combined model of chronic constriction injury (CCI) and intraplantar Complete Freund's Adjuvant (CFA) injection results in enhanced mechanical allodynia, thermal hyperalgesia, a static weight bearing deficit, and notably pronounced spontaneous foot lifting (SFL) behaviour (which under our conditions was not seen in either individual model and may reflect ongoing/spontaneous pain). Dorsal root ganglion (DRG) expression of Activating Transcription Factor-3 (ATF-3), a marker of axonal injury, was no greater in the combined model than CCI alone. Initial pharmacological characterisation of the new model showed that the SFL was reversed by gabapentin or diclofenac, typical analgesics for neuropathic or inflammatory pain respectively, but not by mexiletine, a Na^+ channel blocker effective in both neuropathic and inflammatory pain models. Static weight bearing deficit was moderately reduced by gabapentin, whereas only diclofenac reversed mechanical allodynia. This novel animal model of chronic pain may prove a useful test-bed for further analysing the pharmacological susceptibility of complicated clinical pain states.

1. Introduction

Neuropathic pain is inadequately controlled by current analgesics and progress in strategy for its treatment represents an unmet clinical need. There are a number of rodent models of neuropathic pain, including chronic constriction injury (CCI), a loose ligation of the sciatic nerve that induces intraneural oedema and physical axonal damage (Bennett and Xie, 1988), spinal nerve ligation (Kim and Chung, 1992), partial sciatic nerve ligation (Seltzer et al., 1990) and more recently the spared nerve injury, in which the tibial and common peroneal nerves are cut and ligated and the sural branch of the sciatic nerve is left intact (Decosterd and Woolf, 2000). In addition, specific models of disease-induced neuropathies, such as diabetic neuropathy (Malcangio and Tomlinson, 1998) and post-herpetic neuralgia (Fleetwood-Walker et al., 1999) have been produced. Although some of these models display a modest degree of spontaneous pain-like behaviour (Choi et al., 1994), which is more prominent when there is a concurrence of injured and uninjured afferents (Djouhri et al., 2006; Lee et al., 2003), spontaneous pain remains relatively under-investigated. In addition to the allodynia and hyperalgesia arising from nerve injury, a particularly troublesome feature is spontaneous/paroxysmal pain (Attal and Bouhassira, 2004; Backonja and Stacey, 2004). Chronic inflammatory pain is also problematic clinically. A number of animal models of inflammatory pain have been developed, for example the intraplantar injection of Complete Freund's Adjuvant (CFA; inactivated *Mycobacterium tuberculosis* emulsified in mineral oil) which activates the innate immune system and causes the local release of pro-

inflammatory cytokines (Hargreaves et al., 1988; Iadarola et al., 1988a; Iadarola et al., 1988b; Meller et al., 1994).

Many clinical situations however, do not represent neuropathic or inflammatory pain states in isolation. Such situations may result from severe trauma such as road traffic accident or sporting injury and clinically co-incident nerve damage and inflammation following surgery. Although both clinical neuropathies and laboratory models may involve an immune/inflammatory component at the local site of injury (Said and Hontebeyrie-Joskowicz, 1992), an explicit combined model of peripheral neuropathic pain together with peripheral inflammatory pain could provide new insights into clinically relevant chronic pain states. We hypothesised that such a model might display distinct behavioural or pharmacological properties because inflammatory and neuropathic injuries individually induce different phenotypic changes in afferent neurons (Woolf and Ma, 2007), so the combination may result in a distinct mechanistic profile. Therefore in the present study we introduce a new combined CCI+CFA model, which we hypothesised would bring about an accentuated hypersensitive state, perhaps with distinctive properties. To characterise this combined model we assessed the duration and extent of both evoked and non-evoked pain behaviours, compared to either nerve injury or inflammation alone. In addition we measured the expression of ATF-3, a marker of neuronal injury (Tsujino et al., 2000), in DRG ipsilateral to injury to assess whether nerve damage is exacerbated in the model. We further evaluated the efficacy of the clinically used analgesics, gabapentin, diclofenac and mexiletine (Dworkin et al., 2007; Kingery, 1997) on the behavioural

outcomes to characterise the pharmacology of the model, with the possibility of finding new insights into the treatment of spontaneous pain.

2. **Materials and methods**

2.1. Animals

All experiments were carried out on male Sprague-Dawley rats (Harlan) in accordance with the UK Animals (Scientific Procedures) Act 1986 (and associated guidelines) and had received approval from the University of Edinburgh ethical committee. Animals were given access to food and water *ad libitum* and housed in accordance with Home Office guidelines. Ambient temperature and humidity were 21°C and 50% respectively; lighting was on a schedule of 12 h on: 12 h off, with lights on from 07.00 h to 19.00 h. Rats were normally housed in groups of up to 6 per cage.

2.2. Procedures

Rats (150-200 g) were anaesthetised by inhalation of an isoflurane/O_2 mixture (Zeneca, Cheshire, UK), 4% for induction and 1.5-3% for maintenance. Surgery to produce a chronic constriction injury (CCI) of the sciatic nerve was performed as described previously (Bennett and Xie, 1988). Following hair removal and sterilisation of the skin area (Hibitane 0.05%, Zeneca, UK), a small incision was made on the right hind leg below the pelvis and then the biceps femoris and the gluteus superficialis were carefully separated to expose the sciatic nerve. Upon isolation of the nerve, four loose ligatures were tied around it proximal to the trifurcation using 4-0 chromic catgut (SMI AG, Hunningen, Belgium) with a 1 mm separation between

ligatures. The nerve was then carefully placed back into position and the wound closed with Vicryl sutures (Ethicon UK). For the chronic inflammatory pain model, intraplantar injection of CFA was used (Taurog et al., 1988). Following sterilisation of the plantar surface of the right hindpaw with 0.05% Hibitane, 150 μl of 50% CFA (Sigma; 1 mg *Mycobacterium tuberculosis* in 0.85 ml mineral oil and 0.15 ml mannide mono-oleate) in 0.9% saline was injected between the toes and towards the middle of the paw, avoiding the ankle; the needle was withdrawn slowly to minimise leakage. For the combined CCI+CFA pain model, CFA was injected into the right hindpaw directly after completion of CCI surgery while the animal was still anaesthetised. None of the animals in the present study displayed ventroflexion of the toes or paw eversion in the affected limb, which are reported side-effects of CCI surgery in some laboratories (Nakazato-Imasato and Kurebayashi, 2009).

2.3. Sensory behaviour tests

Animals were habituated to the testing environments and tester twice on separate days for at least 15 min prior to undergoing surgery. Measurements of reflex responses to graded mechanical or thermal stimuli were recorded in conscious animals prior to injury and regularly post-injury to establish a time course of sensitivity. Differences in static weight bearing were also measured, together with spontaneous paw lifting as indicators of non-evoked behaviours that further reflect the experience of pain.

To determine evoked reflex responses to mechanical stimuli, animals were placed on a raised mesh grid and covered with a clear plastic box for

containment. Calibrated von Frey filaments (Stoelting Co, USA) were used to determine the threshold force for withdrawal of the ipsilateral paw compared to the contralateral paw. Filaments were applied to the middle of the plantar surface of each paw from below until the filament bent; this was carried out 8 times per filament, in order of increasing force. The filament at which 50% of the applications resulted in paw withdrawal was recorded as the paw withdrawal threshold (PWT) in grams (g). A withdrawal response was considered valid if the animal's paw was withdrawn from both the testing platform and also away from the filament itself, so as to distinguish from the animal allowing its paw to be simply lifted by the filament. A cut off point was set at a filament bending force of 28 g to prevent tissue damage and this also reduced the possibility of the filament being able to lift the animal's paw.

Thermal hyperalgesia was measured using Hargreaves' plantar test analgesymeter (Hargreaves et al., 1988) (Ugo Basile, Comerio, Italy). Rats were placed in individual plastic boxes on a glass platform and allowed to settle for 5-10 min. An infra-red radiant heat source was positioned under the platform and focused on the middle of the plantar surface of each hindpaw. Following activation, the time in seconds (s) at which the animal withdrew its paw was recorded as paw withdrawal latency (PWL). A cut-off time of 20 s was imposed to avoid possible tissue damage. The test was repeated 3 times for each hindpaw with a 5 min gap between tests to avoid sensitisation.

Reduction in static weight bearing (a test that further reflects aspects of mechanical allodynia) was measured using an incapacitance tester, (Linton Instruments UK); a device that measures the weight distributed to each hind paw individually. Rats were placed in the Perspex container positioned over

force plates with one hind paw on each plate and the weight-bearing force exerted was averaged over 4 s. For each rat, 3 tests were carried out for each paw consecutively at each time point. Results were expressed as the difference between the contralateral and ipsilateral paw reading in grams (g).

We also measured spontaneous foot lifting (SFL), which is thought to be an indicator of ongoing/spontaneous pain, as the only apparent relevant sensory input is mechanical load bearing, which is invariant in these conditions (Bennett and Xie, 1988; Choi et al., 1994; Djouhri et al., 2006). At approximately the same time each day (late morning to early afternoon) rats were placed in individual plastic observation boxes in which their movement was unrestricted. No evoked behaviour tests were carried out before the SFL assessment to avoid the possibility of any element of hypersensitivity developing due to testing. The cumulative duration of lifting of the ipsilateral hindpaw was recorded over a single period of 3 min. Foot lifting associated with locomotion, grooming or body repositioning was excluded. Deliberate spontaneous lifting of the affected paw, sometimes associated with overt pain-like behaviour such as flicking or paw licking was scored for all of the time that the paw was raised from the surface. The occurrence of the SFL behaviour was confirmed by an independent observer. No discernible SFL behaviour was noted for the contralateral hindlimb throughout the study.

2.4. Immunohistochemistry

Ten days after surgery, three rats from each group (CCI, CFA and combined CCI + CFA) were deeply anaesthetised with sodium pentabarbitone

(Euthatal, Merial Animal Health Ltd) and transcardially perfused with 0.9% sodium chloride followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline. The L4 and L5 DRG were removed, post-fixed overnight and then transferred to 5% sucrose for 1 h, 10% sucrose for 3 h and 30% sucrose overnight. DRGs were then embedded in OCT compound (Cellpath Ltd), frozen in isopentane on dry ice and 10 μm sections cut on a Leica CM1950 cryostat. Sections were mounted on ten polylysine-coated glass slides (VWR) with each serial section mounted on a consecutive slide from its neighbouring section, so that each section on a particular slide was >100 μm from the next one. This prevented the same neuron from being counted twice on one slide. Following washing in PBS and blocking with 10% normal goat serum and 4% fish skin gelatin in PBS containing 0.2% Tween-20, the sections were labelled overnight with rabbit anti-ATF-3 (1:250, Santa Cruz) in the same medium but with the goat serum concentration at 4%. ATF-3 antibody binding was visualised using pre-absorbed goat anti-rabbit Alexa-568 (Invitrogen) secondary antibody. Sections were counterstained with mouse anti-NF-200, (1:1000, Sigma), a marker of myelinated neurons and visualised with goat anti-mouse Alexa-488 secondary antibody. Images were taken at 20x magnification using a Leica DM2500 fluorescence microscope equipped with a Leica DFC310 camera and analysed using ImageJ software. Three animals were used for each condition and a minimum of 4 images from each animal were analysed.

2.5. Drugs and treatments

We examined the effects of gabapentin (Ascent Scientific), diclofenac (Sigma) and mexiletine (Sigma) on the combined model of nerve injury and inflammation. All drugs were dissolved in 0.9% sterile saline (Sigma) and administered by a single intraperitoneal injection using the following doses: gabapentin 50 mg/kg, diclofenac 100 mg/kg and mexiletine 30 mg/kg. The doses we administered were chosen on the basis of effective analgesic doses used in common models of neuropathic or inflammatory pain (Erichsen and Blackburn-Munro, 2002; Laird et al., 2001; Nagakura et al., 2003; Nakazato-Imasato et al., 2009; Pedersen and Blackburn-Munro, 2006; Wallace et al., 2007). Drugs were administered 8 days following CCI surgery/CFA injection and behavioural testing was carried out 90, 180 and 300 min after injection (n=8 per group). Animals were randomly placed into drug or vehicle groups following baseline testing and testing was carried out blinded.

2.6. Statistical Analyses

For behavioural measures, all values were calculated as group mean ± SEM at each time point. Thermal hyperalgesia (PWL) was analysed by comparing ipsilateral to contralateral values over time and also to pre-surgery baseline, using two-way repeated measures ANOVA with Bonferroni's *post hoc* test. As the von Frey filaments used to measure mechanical allodynia have constant logarithmic intervals between their bending forces, logarithmic-transformed (y=ln(y)) values were used to normalise data for analysis, enabling two-way repeated measures ANOVA (with Bonferroni's *post hoc* test) as for thermal data. Injury-induced alteration of ipsilateral static weight bearing, as compared to pre-surgery baseline was analysed using repeated

measures ANOVA with Dunnett's *post hoc* test, and each model was compared to the others using a two-way repeated measures ANOVA with Bonferroni's *post hoc* test. Mean duration of spontaneous ipsilateral foot lifting (SFL) was analysed using two-way repeated measures ANOVA with Bonferroni's *post hoc* test. ATF-3 immunostaining was analysed using a one-way ANOVA followed by Newman-Keuls multiple comparison *post hoc* test. The effects of pharmacological agents on ipsilateral mechanical allodynia compared to pre-drug values were assessed by repeated measures ANOVA (Friedman test) followed by Dunn's *post hoc* test. Effects on SFL duration and static weight bearing difference were analysed by one-way repeated measures ANOVA followed by Dunnett's *post hoc* test to compare pre-drug values with post-drug values. The percentage of rats exhibiting an SFL duration of >10 s was analysed using Fisher's exact test. All data evaluations were carried out using Graphpad Prism software and in each case a value of $p<0.05$ was taken to indicate statistical significance.

3. Results

3.1. Thermal hyperalgesia and mechanical allodynia in a combined model of neuropathic and inflammatory pain compared to nerve injury or inflammation alone.

In the Hargreaves' thermal test, the combined CCI+CFA model induced sensitisation compared to baseline and to the contralateral limb, that was longer in duration than in either of the single models (Fig. 1 a-c, n=6). Paw withdrawal latency (PWL) in response to thermal stimuli was significantly

decreased in the injured hindpaw compared to pre-surgery baseline and also relative to the contralateral paw as determined by two-way repeated measures ANOVA, comparing the effect of treatment over time (Treatment effect; CFA; $F=16.62$ $p=0.0022$, CCI; $F=17.74$, $p=0.0019$ CCI+CFA; $F=29.85$, $p=0.0003$). PWL in the ipsilateral paw of the combined CCI+CFA model remained significantly different from baseline for up to 28 days post injury and for up to 14 days compared to the contralateral paw, outlasting any differences in the single injury models, and reflecting a longer lasting thermal hyperalgesia.

The combined CCI + CFA injury also resulted in long-lasting mechanical allodynia (Fig. 1 d-f) which outlasted the effect of nerve injury alone. Although the duration of mechanical allodynia was not discernibly different between CFA and the combined CCI + CFA model, the data show that the ipsilateral sensitisation following the combined model was of a greater magnitude than in either of the single models (Fig. 1 d-f). To compare directly the degree of allodynia we analysed the difference in PWT (contralateral-ipsilateral) following injury and revealed a significant difference in the interaction between treatment and time through the different injury groups (two-way repeated measures ANOVA, $F=2.34$, $p=0.002$, following successful tests for normality), with individual group differences identified by Bonferroni *post hoc* tests occurring between CCI and the combined CCI + CFA model at days 6 ($p<0.001$) and 7 ($p<0.05$), and between CFA and the combined CCI + CFA model at days 6 ($p<0.001$) and 7 ($p<0.01$, $n=6$ rats per group). There were no time points where significant differences were identified between the CCI and CFA groups. Mechanical allodynia in the combined model remained

statistically significant for 56 days compared to baseline and 49 days compared to the contralateral paw, with corresponding values for CCI alone being 49 and 21 days respectively. Taken together, these data indicate that the combined CCI+CFA model displays a prolonged time course and/or greater magnitude of hypersensitivity in evoked responses than either of the single component injuries.

3.2. Static weight bearing difference in a combined model of neuropathic and inflammatory pain compared to nerve injury or inflammation alone.

Static weight bearing measurements showed a difference between the ipsilateral and contralateral hind paw from day 1 post-surgery in all three models, as evaluated by repeated measures ANOVA with Dunnett's *post hoc* test (Fig. 2 a). In both the combined CCI + CFA model and the CCI model the static weight bearing difference was significantly different from baseline levels at all time points following injury (combined model: $p<0.01$ at days 1, 13, 15 and 17 and $p<0.001$ at days 2, 3, 6, and 9; CCI: $p<0.05$ at day 17, $p<0.01$ at day 15 and $p<0.001$ at days 1, 2, 3, 6, 9 and 13). After CFA injection, static weight bearing difference was shorter lasting, being significantly different from baseline only at days 1, 2 and 6 ($p<0.05$ at day 1, $p<0.01$ at days 2 and 6). There was no significant difference between the combined CCI + CFA model and CCI alone. Both CCI and the combined CCA + CFA model resulted in a consistently greater ipsilateral-contralateral weight bearing difference than CFA alone. They both showed significantly greater static weight bearing difference compared to CFA alone, which was statistically significant at days 3, 9, 13 and 15 post injury for the combined CCI + CFA model (all $p<0.01$)

and at days 3, 9 and 13 for CCI ($p<0.01$ at days 3 and 9 and $p<0.001$ at day 13) (two-way repeated measures ANOVA with Bonferroni's *post hoc* test).

3.3. Spontaneous foot lifting in a combined model of neuropathic and inflammatory injury compared to nerve injury or inflammation alone.

Rats with combined CCI + CFA injury displayed significantly more spontaneous pain-like behaviour (spontaneous ipsilateral paw lifting; SFL), compared to those with nerve injury or inflammation alone. This was quantified as the cumulative time over which each rat raised its injured paw throughout a 3 min period (Fig. 2 b) and was analysed by two-way repeated measures ANOVA with Bonferroni's *post hoc* test. SFL was significantly greater in the combined CCI + CFA model compared to CCI alone at days 8 and 9 post-surgery ($p<0.001$) and also significantly greater compared to CFA alone at days 7, 8 and 9 post-surgery ($p<0.05$ at day 7 and $p<0.001$ at days 8 and 9). No SFL of the contralateral paw was observed. In our experiments, only very low levels of SFL were observed ipsilateral to CCI or CFA injury alone, which did not reach statistical significance. In our view it seems likely that SFL may only be observed when hypersensitivity is at its greatest magnitude, so this may well be detectable only over a shorter period than the statistically significant enhancement of von Frey responses.

3.4. ATF-3 expression in DRG in a combined model of neuropathic and inflammatory pain compared to nerve injury or inflammation alone.

As ATF-3 expression in DRG neurons is considered to reflect explicit injury to their axons, as opposed to inflammation (Tsujino et al., 2000), we

used immunofluorescence histochemistry to assess its expression in each of the three different pain models (Fig. 3). ATF-3-positive cells from at least 4 non-adjacent transverse sections (10 μm) of L4 and L5 DRG per animal (n=3 per group) were counted, and expressed as a % of the total number of cells counted in these sections. DRG neurons showed significant ATF-3 expression following nerve injury or nerve injury plus inflammation, but not following inflammation alone. One-way ANOVA (with Student-Newman-Keuls *post hoc* test for all pair-wise comparisons) indicated that ATF-3 expression was significantly higher after CCI ($p<0.001$) or combined CCI + CFA model ($p<0.001$) compared to CFA. ATF-3 expression in DRG cells was not significantly different ($p=0.091$) between CCI alone and the combined CCI + CFA model.

3.5. Effects of gabapentin, diclofenac and mexiletine on behavioural measures of sensitisation in a combined model of neuropathic and inflammatory pain

Three analgesic agents with different modes of action and preferential influences were assessed. Gabapentin (effective in neuropathic pain), diclofenac (effective in inflammatory pain) and mexiletine (a broad specificity Na^+ channel blocker) were examined for their effects on mechanical allodynia, static weight bearing difference and SFL in the combined CCI + CFA model of neuropathic and inflammatory pain at eight days post-surgery (Fig. 4 and 5). After a single (IP) dose, all three agents showed some attenuation of the ipsilateral mechanical allodynia, although only diclofenac showed a significant reversal compared to its pre-drug value ($p<0.05$ at 300 min post-

administration, repeated measures ANOVA (Friedman test) followed by Dunn's *post hoc* test) (Fig. 4 a).

The CCI + CFA-induced weight bearing difference between the ipsilateral and contralateral paws was significantly reduced only by gabapentin at 90 min post-dosing compared to pre-drug values, $p<0.05$, as shown by one-way repeated measures ANOVA followed by Dunnett's *post hoc* test (Fig. 4 b).

The ipsilateral SFL that was specifically induced by the combined CCI + CFA model was significantly attenuated by gabapentin and diclofenac, while mexiletine or vehicle had no discernible effect (Fig. 5 a-d). Both gabapentin and diclofenac significantly reduced the percentage of rats exhibiting >10 s of SFL at 90 and 180 min post-drug dose (Fig. 5 b and c) ($p<0.001$, Fisher's exact test). When the data were analysed as mean cumulative SFL, gabapentin showed a significant reduction at 180 min post-dose compared to pre-drug values (one-way repeated measures ANOVA followed by Dunnett's multiple comparison test).

4. Discussion

The newly introduced CCI + CFA rodent model of chronic pain, which was designed to reflect the consequences of traumatic injuries encountered clinically, results in greater or more prolonged mechanical allodynia and thermal hyperalgesia compared to the component models alone. It also elicits spontaneous foot lifting (SFL), a non-evoked behaviour that we did not observe in either of the CCI or CFA individual models and may reflect ongoing

or spontaneous pain. The additional effects seen in the mechanical and thermal sensitivity do not however translate to further changes in static weight bearing, where we observed no significant difference between CCI + CFA and CCI alone. The expression levels of ATF-3 in DRG, reflecting frank neuronal injury, were no different between the combined CCI + CFA model and CCI alone suggesting that the enhanced sensitivity observed in some tests with the CCI + CFA model is not due to a greater degree of nerve damage. We carried out a preliminary investigation of the effects of some established analgesic agents on various behaviours in the combined CCI + CFA model. In these experiments we focused on tests that relate to mechanical sensation: von Frey (evoked mechanical response), static weight-bearing difference (non-evoked mechanical response) and SFL but did not assess thermal responses. This was because in the newly observed SFL behaviour there will be some potentially relevant input through mechanosensitive afferents prior to the foot lift even though it is not being altered by external events. Acute, single dose, administration of all three analgesics tested showed a trend towards reversal of mechanical allodynia in the von Frey filament test, although only the effect of diclofenac reached statistical significance. Only gabapentin showed a significant effect on static weight bearing difference. Although the static weight-bearing test reflects in part aspects of mechanical allodynia like the von Frey filament test, it differed by not showing a statistically significant effect of diclofenac. This may be because the ongoing weight-bearing test reflects in part different mechanosensitive afferents from those activated in the transient von Frey test and is also affected to some extent by proprioceptive and motivational factors, resulting in a tendency to show

greater variability. Although not statistically significant with the current data set, diclofenac did cause a mean 25% attenuation of weight-bearing difference at 300 min. It is possible that in further experiments with a larger number of replicates that this would reach statistical significance, thereby resolving any apparent disparity in the pharmacological sensitivity of the von Frey and weight-bearing tests. Both gabapentin and diclofenac but not mexiletine significantly reduced SFL behaviour. Thus, particular facets of the CCI + CFA-induced pain state appear to show differential sensitivity to analgesics of different types.

Animal models of clinical pain seek to improve understanding of the aetiology of persisting pain states and also to identify potential targets for the development of analgesics. There is a continuing need for animal models that best represent clinical conditions, both in terms of duration and the spectrum of spontaneous and evoked responses observed. Our novel CCI + CFA model was designed to encompass both neuropathic and inflammatory injuries, which often occur together following severe trauma. In this model, both evoked and non-evoked measures of pain behaviour were enhanced in the ipsilateral hindpaw. Clear differences in weight bearing between ipsilateral and contralateral hindlimbs were seen in all models but this was greater and longer lasting in the CCI and combined CCI + CFA models compared to the CFA alone. The CCI + CFA model provides a robust and long-lasting pain state which displays not only symptoms such as hypersensitivity to mechanical and thermal stimuli, but also non-evoked raising of the injured paw, which is considered to be an indicator of spontaneous pain (Bennett and Xie, 1988; Choi et al., 1994; Djouhri et al., 2006). Spontaneous pain is a major

clinical problem, which is not sufficiently represented in current basic science studies, where many of the sensory behavioural tests are focused on evoked withdrawal responses, when in fact 96% of clinical neuropathies display ongoing, spontaneous pain. Although still representing important clinical problems, only 64% and 38% of neuropathic pain cases have a mechanical or thermal hypersensitivity component, respectively) (Backonja and Stacey, 2004).

The molecular mechanisms that could underlie the increased sensitivities to mechanical and thermal stimuli, and also the spontaneous pain component observed in this model remain to be clarified. However, previous work has suggested that spontaneous pain is associated with unprovoked firing in both unmyelinated and myelinated fibres (Djouhri et al., 2006). Djouhri and colleagues described how an L5 spinal-nerve axotomy, either alone or in combination with loose ligation of the L4 spinal nerve, both produce spontaneous activity in intact nociceptive C-fibres but only the latter is associated with SFL behaviour; thus emphasising the importance of co-existing injured and uninjured afferents in producing hypersensitivity. Djouhri et al. also reported a correlation between the rate of spontaneous firing of intact C-fibres and the extent of SFL. These findings may mirror the greater degree of spontaneous pain behaviour observed in our CCI + CFA model. Unlike some other reports (Attal et al., 1990; Bennett and Xie, 1988; Dowdall et al., 2005) we observed no significant SFL following CCI alone. The difference may be due to the relative intensity of the ligation injury applied in different laboratories. Inflammatory mediators, such as interleukins and TNF-α are released during Wallerian degeneration of damaged nerves (Shamash et

al., 2002) and following inflammation (Safieh-Garabedian et al., 1995) and have been linked with the appearance of spontaneous activity in DRG neurons (Schafers et al., 2003). Furthermore, the up-regulation of NGF, induced in response to TNF-α following peripheral inflammation (Woolf et al., 1997), has also been shown to lead to spontaneous activity in DRG neurons. Although the site of peripheral inflammation in the CCI + CFA model is not at the nerve trunk, the combination of the peri-neural inflammation associated with CCI, and peripheral inflammation following CFA may exert a cumulative effect, which might cause an increase in the spontaneous activity of nociceptive C-fibres to the levels necessary to generate greater spontaneous pain-like behaviour, such as SFL.

An important consideration in evaluating such a mixed neuropathic/inflammatory pain model is that either of these injuries independently induces a characteristic and quite distinct set of phenotypic changes in DRG cells (Woolf and Ma, 2007). The combined model is therefore likely to represent the integrated outcome; not only from processes changing the releasable neuropeptide content of particular afferents but also from processes elevating expression of the original neuropeptides and trophic factors. The resulting balance of influence on central processing that appears to engender SFL clearly represents a complex matrix that will need to be further elucidated in order to fully understand the basis of such spontaneous pain-like behaviours.

To determine the extent of neuronal damage following the CCI + CFA model, expression of the nerve injury marker ATF-3 (Tsujino et al., 2000) in DRG neurons was compared to the single injury pain models. We found that

the increased nociceptive sensitisation associated with the CCI + CFA model was not due to a greater degree of nerve injury, as the expression of ATF-3 was similar in DRG of CCI + CFA and CCI alone. There was little or no ATF-3 expression in response to CFA, in agreement with other studies (Palm et al., 2008; Segond von Banchet et al., 2009). These findings indicate that the level of ATF-3 expression in DRG is not a critical determinant of the SFL behaviour that was essentially observed only in the CCI + CFA model. In further studies it would be of interest to carry out an immunohistochemical assessment of neuropeptide expression in dorsal root ganglia of CCI + CFA animals compared to the individual models because neuropathic and inflammatory injuries induce distinct phenotypic changes (Woolf and Ma, 2007). Such an extensive survey was however beyond the scope of the present work.

Both gabapentin and diclofenac significantly reduced CCI + CFA-induced SFL behaviour. Gabapentin is thought to act by binding to $\alpha 2\delta$-1 or $\alpha 2\delta$-2 subunits of voltage-gated Ca^{2+} channels (Marais et al., 2001), which associate with the $\alpha 1$ subunit to regulate current amplitude. Although gabapentin is generally considered as an analgesic for neuropathic pain states (Dworkin et al., 2007), it dose-dependently reduces C-fibre-evoked responses of neurons recorded following carrageenan-induced inflammation (Stanfa et al., 1997). Diclofenac is a well-used non-steroidal anti-inflammatory (NSAID) drug, being a broad-spectrum cyclo-oxygenase (COX) inhibitor with modest selectivity for COX-2 over COX-1 (Giuliano and Warner, 1999). Eicosanoid inflammatory mediators, eg prostaglandins, could therefore play a role in bringing about SFL behaviour. It has been reported that prostaglandin-regulated descending control from the PAG preferentially targets C-nociceptor

evoked activity (Leith et al., 2007), raising the possibility that a component of the antinociceptive effect of diclofenac here could be centrally mediated. Pain state-induced hypersensitivity of dorsal horn neurons is reversed by Na^+ channel blockers (Blackburn-Munro and Fleetwood-Walker, 1997), so it was unexpected that mexiletine, a sodium channel blocker, efficacious in both neuropathic and inflammatory pain (Akada et al., 2006; Dworkin et al., 2007; Erichsen et al., 2003; Laird et al., 2001; Nakazato-Imasato et al., 2009) did not reduce SFL here, despite a typically effective dose being used. We cannot exclude of course the possibility that different doses and chronic treatment may be effective. It has been reported however that in patients with peripheral nerve injury, mexiletine is less effective at reversing spontaneous pain than mechanically evoked pain (Wallace et al., 2000). It is possible that this is because spontaneous pain reflects a greater degree of underlying hypersensitivity but we were not able to investigate this further in the present study. Mexiletine, which is orally active, and its congener lidocaine are both effective analgesics when systemically administered, although their therapeutic margins are narrow (Attal et al., 2004; Jarvis and Coukell, 1998). Both act primarily as Na^+ channel blockers that are not selective for channel subtypes, with mexiletine displaying slightly higher potency at both tetrodotoxin-sensitive and –insensitive channels (Weiser, 2006). We did not therefore examine the effects of systemically administered lidocaine. Neither did we assess the effects of regional administration of lidocaine at high concentrations to produce local anaesthesia because the main (sciatic) nerve from the distal hindlimb contains both afferent and efferent fibres, so any

directed attempt at local anaesthesia would be likely to compromise the animal's ability to deliver an effective reflex withdrawal.

All three analgesics tested showed a trend towards reduction in mechanical allodynia in the CCI + CFA model, although it was only statistically significant with diclofenac, which could be a result of it attenuating both peri-neural and peripheral inflammation.

The static weight bearing difference in the CCI + CFA model here was significantly reduced by gabapentin, contrasting with observations in CCI alone and other nerve injury models where the close analogue, (1S, 3R)-3-methylgabapentin failed to affect static weight-bearing at doses causing robust reversal of mechanical allodynia (Nakazato-Imasato and Kurebayashi, 2009; Urban et al., 2005). Gabapentin has also been shown to reduce static weight bearing difference in a rat model of osteoarthritis, whereas a COX-2 inhibitor did not (Ivanavicius et al., 2007). However, in another study following CFA injection, diclofenac was reported to successfully reverse static weight bearing differences (Huntjens et al., 2009). It is clear from the present study, which represents a new model of spontaneous pain, that different facets of ongoing pain states, assessed in different behavioural tests, are differentially susceptible to putative analgesic agents. This emphasises the need for a wide-ranging behavioural assessment in any studies evaluating the likely clinical efficacy of new analgesic agents.

In summary, we have introduced a novel, clinically relevant model of chronic pain, which combines both neuropathic and inflammatory injury. This model could prove useful as a new tool for investigating the mechanisms of

spontaneous pain, a common clinical complaint. The SFL that is characteristic of this model is sensitive to two clinically used analgesics that are thought to preferentially target neuropathic and inflammatory pain, suggesting that combinations of such agents may be useful in the treatment of clinically relevant human pain states. However, it remains to be ascertained whether combined use of such agents leads to greater analgesic efficacy in a clinical context. The CCI + CFA model could well contribute to identifying new therapeutic targets for analgesia in chronic pain and to improving understanding of the complex matrix of molecular changes occurring in DRG and spinal cord that may help to define new strategies for the relief of chronic pain.

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Disclosures

The authors have no conflict of interest to declare.

References

Akada, Y., Ogawa, S., Amano, K., Fukudome, Y., Yamasaki, F., Itoh, M., Yamamoto, I., 2006. Potent analgesic effects of a putative sodium channel blocker M58373 on formalin-induced and neuropathic pain in rats. Eur J Pharmacol 536, 248-255.

Attal, N., Bouhassira, D., 2004. Can pain be more or less neuropathic? Pain 110, 510-511.

Attal, N., Jazat, F., Kayser, V., Guilbaud, G., 1990. Further evidence for 'pain-related' behaviours in a model of unilateral peripheral mononeuropathy. Pain 41, 235-251.

Attal, N., Rouaud, J., Brasseur, L., Chauvin, M., Bouhassira, D., 2004. Systemic lidocaine in pain due to peripheral nerve injury and predictors of response. Neurology 62, 218-225.

Backonja, M.M., Stacey, B., 2004. Neuropathic pain symptoms relative to overall pain rating. J Pain 5, 491-497.

Bennett, G.J., Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33, 87-107.

Blackburn-Munro, G., Fleetwood-Walker, S.M., 1997. The effects of Na+ channel blockers on somatosensory processing by rat dorsal horn neurones. Neuroreport 8, 1549-1554.

Choi, Y., Yoon, Y.W., Na, H.S., Kim, S.H., Chung, J.M., 1994. Behavioral signs of ongoing pain and cold allodynia in a rat model of neuropathic pain. Pain 59, 369-376.

Decosterd, I., Woolf, C.J., 2000. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. Pain 87, 149-158.

Djouhri, L., Koutsikou, S., Fang, X., McMullan, S., Lawson, S.N., 2006. Spontaneous pain, both neuropathic and inflammatory, is related to frequency of spontaneous firing in intact C-fiber nociceptors. J Neurosci 26, 1281-1292.

Dowdall, T., Robinson, I., Meert, T.F., 2005. Comparison of five different rat models of peripheral nerve injury. Pharmacol Biochem Behav 80, 93-108.

Dworkin, R.H., O'Connor, A.B., Backonja, M., Farrar, J.T., Finnerup, N.B., Jensen, T.S., Kalso, E.A., Loeser, J.D., Miaskowski, C., Nurmikko, T.J., Portenoy, R.K., Rice, A.S., Stacey, B.R., Treede, R.D., Turk, D.C., Wallace, M.S., 2007. Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 132, 237-251.

Erichsen, H.K., Blackburn-Munro, G., 2002. Pharmacological characterisation of the spared nerve injury model of neuropathic pain. Pain 98, 151-161.

Erichsen, H.K., Hao, J.X., Xu, X.J., Blackburn-Munro, G., 2003. A comparison of the antinociceptive effects of voltage-activated Na+ channel blockers in two rat models of neuropathic pain. Eur J Pharmacol 458, 275-282.

Fleetwood-Walker, S.M., Quinn, J.P., Wallace, C., Blackburn-Munro, G., Kelly, B.G., Fiskerstrand, C.E., Nash, A.A., Dalziel, R.G., 1999. Behavioural changes in the rat following infection with varicella-zoster virus. J Gen Virol 80 (Pt 9), 2433-2436.

Giuliano, F., Warner, T.D., 1999. Ex vivo assay to determine the cyclooxygenase selectivity of non-steroidal anti-inflammatory drugs. Br J Pharmacol 126, 1824-1830.

Hargreaves, K., Dubner, R., Brown, F., Flores, C., Joris, J., 1988. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32, 77-88.

Huntjens, D.R., Spalding, D.J., Danhof, M., Della Pasqua, O.E., 2009. Differences in the sensitivity of behavioural measures of pain to the selectivity of cyclo-oxygenase inhibitors. Eur J Pain 13, 448-457.

Iadarola, M.J., Brady, L.S., Draisci, G., Dubner, R., 1988a. Enhancement of dynorphin gene expression in spinal cord following experimental inflammation: stimulus specificity, behavioral parameters and opioid receptor binding. Pain 35, 313-326.

Iadarola, M.J., Douglass, J., Civelli, O., Naranjo, J.R., 1988b. Differential activation of spinal cord dynorphin and enkephalin neurons during hyperalgesia: evidence using cDNA hybridization. Brain Res 455, 205-212.

Ivanavicius, S.P., Ball, A.D., Heapy, C.G., Westwood, F.R., Murray, F., Read, S.J., 2007. Structural pathology in a rodent model of osteoarthritis is associated with neuropathic pain: increased expression of ATF-3 and pharmacological characterisation. Pain 128, 272-282.

Jarvis, B., Coukell, A.J., 1998. Mexiletine. A review of its therapeutic use in painful diabetic neuropathy. Drugs 56, 691-707.

Kim, S.H., Chung, J.M., 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50, 355-363.

Kingery, W.S., 1997. A critical review of controlled clinical trials for peripheral neuropathic pain and complex regional pain syndromes. Pain 73, 123-139.

Laird, J.M., Carter, A.J., Grauert, M., Cervero, F., 2001. Analgesic activity of a novel use-dependent sodium channel blocker, crobenetine, in mono-arthritic rats. Br J Pharmacol 134, 1742-1748.

Lee, D.H., Iyengar, S., Lodge, D., 2003. The role of uninjured nerve in spinal nerve ligated rats points to an improved animal model of neuropathic pain. Eur J Pain 7, 473-479.

Leith, J.L., Wilson, A.W., Donaldson, L.F., Lumb, B.M., 2007. Cyclooxygenase-1-derived prostaglandins in the periaqueductal gray differentially control C- versus A-fiber-evoked spinal nociception. J Neurosci 27, 11296-11305.

Malcangio, M., Tomlinson, D.R., 1998. A pharmacologic analysis of mechanical hyperalgesia in streptozotocin/diabetic rats. Pain 76, 151-157.

Marais, E., Klugbauer, N., Hofmann, F., 2001. Calcium channel alpha(2)delta subunits-structure and Gabapentin binding. Mol Pharmacol 59, 1243-1248.

Meller, S.T., Cummings, C.P., Traub, R.J., Gebhart, G.F., 1994. The role of nitric oxide in the development and maintenance of the hyperalgesia produced by intraplantar injection of carrageenan in the rat. Neuroscience 60, 367-374.

Nagakura, Y., Okada, M., Kohara, A., Kiso, T., Toya, T., Iwai, A., Wanibuchi, F., Yamaguchi, T., 2003. Allodynia and hyperalgesia in adjuvant-induced arthritic rats: time course of progression and efficacy of analgesics. J Pharmacol Exp Ther 306, 490-497.

Nakazato-Imasato, E., Kurebayashi, Y., 2009. Pharmacological characteristics of the hind paw weight bearing difference induced by chronic constriction injury of the sciatic nerve in rats. Life Sci 84, 622-626.

Nakazato-Imasato, E., Tanimoto-Mori, S., Kurebayashi, Y., 2009. Effect of mexiletine on dynamic allodynia induced by chronic constriction injury of the sciatic nerve in rats. J Vet Med Sci 71, 991-994.

Palm, F., Mossner, R., Chen, Y., He, L., Gerlach, M., Bischofs, S., Riederer, P., Lesch, K.P., Sommer, C., 2008. Reduced thermal hyperalgesia and enhanced peripheral nerve injury after hind paw inflammation in mice lacking the serotonin-transporter. Eur J Pain 12, 790-797.

Pedersen, L.H., Blackburn-Munro, G., 2006. Pharmacological characterisation of place escape/avoidance behaviour in the rat chronic constriction injury model of neuropathic pain. Psychopharmacology (Berl) 185, 208-217.

Safieh-Garabedian, B., Poole, S., Allchorne, A., Winter, J., Woolf, C.J., 1995. Contribution of interleukin-1 beta to the inflammation-induced increase in nerve growth factor levels and inflammatory hyperalgesia. Br J Pharmacol 115, 1265-1275.

Said, G., Hontebeyrie-Joskowicz, M., 1992. Nerve lesions induced by macrophage activation. Res Immunol 143, 589-599.

Schafers, M., Lee, D.H., Brors, D., Yaksh, T.L., Sorkin, L.S., 2003. Increased sensitivity of injured and adjacent uninjured rat primary sensory neurons to exogenous tumor necrosis factor-alpha after spinal nerve ligation. J Neurosci 23, 3028-3038.

Segond von Banchet, G., Boettger, M.K., Fischer, N., Gajda, M., Brauer, R., Schaible, H.G., 2009. Experimental arthritis causes tumor necrosis factor-alpha-dependent infiltration of macrophages into rat dorsal root ganglia which correlates with pain-related behavior. Pain 145, 151-159.

Seltzer, Z., Dubner, R., Shir, Y., 1990. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 43, 205-218.

Shamash, S., Reichert, F., Rotshenker, S., 2002. The cytokine network of Wallerian degeneration: tumor necrosis factor-alpha, interleukin-1alpha, and interleukin-1beta. J Neurosci 22, 3052-3060.

Stanfa, L.C., Singh, L., Williams, R.G., Dickenson, A.H., 1997. Gabapentin, ineffective in normal rats, markedly reduces C-fibre evoked responses after inflammation. Neuroreport 8, 587-590.

Taurog, J.D., Argentieri, D.C., McReynolds, R.A., 1988. Adjuvant arthritis. Methods Enzymol 162, 339-355.

Tsujino, H., Kondo, E., Fukuoka, T., Dai, Y., Tokunaga, A., Miki, K., Yonenobu, K., Ochi, T., Noguchi, K., 2000. Activating transcription factor 3 (ATF3) induction by axotomy in sensory and motoneurons: A novel neuronal marker of nerve injury. Mol Cell Neurosci 15, 170-182.

Urban, M.O., Ren, K., Park, K.T., Campbell, B., Anker, N., Stearns, B., Aiyar, J., Belley, M., Cohen, C., Bristow, L., 2005. Comparison of the antinociceptive profiles of gabapentin and 3-methylgabapentin in rat models of acute and persistent pain: implications for mechanism of action. J Pharmacol Exp Ther 313, 1209-1216.

Wallace, M.S., Magnuson, S., Ridgeway, B., 2000. Efficacy of oral mexiletine for neuropathic pain with allodynia: a double-blind, placebo-controlled, crossover study. Reg Anesth Pain Med 25, 459-467.

Wallace, V.C., Segerdahl, A.R., Lambert, D.M., Vandevoorde, S., Blackbeard, J., Pheby, T., Hasnie, F., Rice, A.S., 2007. The effect of the

palmitoylethanolamide analogue, palmitoylallylamide (L-29) on pain behaviour in rodent models of neuropathy. Br J Pharmacol 151, 1117-1128.

Weiser, T., 2006. Comparison of the effects of four Na+ channel analgesics on TTX-resistant Na+ currents in rat sensory neurons and recombinant Nav1.2 channels. Neurosci Lett 395, 179-184.

Woolf, C.J., Allchorne, A., Safieh-Garabedian, B., Poole, S., 1997. Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor alpha. Br J Pharmacol 121, 417-424.

Woolf, C.J., Ma, Q., 2007. Nociceptors--noxious stimulus detectors. Neuron 55, 353-364.

Figure Legends

Fig. 1. Time-courses of thermal hyperalgesia (a-c) and mechanical allodynia (d-f) following CFA inflammation (a and d), CCI nerve injury (b and e) or combined CCI + CFA (c and f). Responses are shown for ipsilateral (□) and contralateral (■) hindpaws. Day of surgery is day 0 and pre-surgery baseline values are indicated as B/L. Data are expressed as mean ± SEM for ipsilateral and contralateral paw withdrawal latency (PWL) measured in s, and as transformed (y=ln(y)) data for paw withdrawal threshold (PWT) measured in g. Two-way repeated measures ANOVA with Bonferroni's *post hoc* test was used to compare post-surgery values for ipsilateral and contralateral hindpaws to pre-surgery baselines (*p<0.05, **p<0.01, ***p<0.001), or to compare ipsilateral to contralateral values over time (#p<0.05, ##p<0.01, ###p<0.001), n=6.

Fig. 2. Time course of: a) static weight bearing difference and b) spontaneous foot lifting (SFL) following CFA (white columns), CCI (grey columns), or combined CCI + CFA (black columns). Surgery was performed at Day 0, and data are shown as mean ± SEM (n=8) for the difference between the ipsilateral and contralateral hindpaws or the cumulative foot lifting duration (in s) over a three min period. In a) two-way repeated measures ANOVA with Bonferroni's *post hoc* test revealed **$p<0.01$ for combined CCI + CFA compared to CCI, ## and ### $p<0.01$ and $p<0.001$ respectively for combined model compared to CFA. In b) two-way repeated measures ANOVA with Bonferroni *post hoc* test revealed ***$p<0.001$ for combined model compared to CCI, as well as # and ### $p<0.05$ and $p<0.001$ respectively for combined CCI + CFA compared to CFA.

Fig. 3. Typical immunofluorescence images of ATF-3 expression in the DRG following, a) CFA, b) CCI and c) combined CCI + CFA. ATF-3 staining is clearly punctate and confined to the nucleus (shown as intense compact staining, or in red). The marker of myelinated neurons, NF-200 is shown as diffuse cytosolic staining or in green. d) The percentage of ATF-3-positive neurons in L4 and L5 DRGs ten days after surgery is shown. Statistical analysis by one-way ANOVA with Newman-Keuls multiple comparison test revealed ***$p<0.001$ for CCI compared to CFA and for combined CCI + CFA compared to CFA.

Fig. 4. The effect of analgesics on: a) mechanical allodynia and b) differences in static weight bearing in the combined CCI + CFA model, 8 days post-surgery. The drugs administered systemically (IP) were gabapentin 50 mg/kg (●), diclofenac 100 mg/kg (▲), mexiletine 30 mg/kg (▼) or vehicle 0.9% saline (■). Data are expressed as the mean PWT ± SEM in g from the ipsilateral, sensitised side. Repeated measures ANOVA (Friedman's test) followed by Dunn's *post hoc* test revealed * $p<0.05$ for diclofenac at 300 min compared to pre-drug values. In b) data are expressed as the mean percentage of the pre-drug static weight bearing difference ± SEM (static weight bearing difference = contralateral minus ipsilateral in g). One-way repeated measures ANOVA followed by Dunnett's multiple comparison test revealed *$p<0.05$ for gabapentin at 90 min compared to pre-drug values.

Fig. 5. The effects of analgesics (administered IP) on spontaneous foot lifting (SFL) behaviour in the combined CCI + CFA model at 8 days post-surgery: a) Vehicle (0.9% saline), b) Gabapentin (50 mg/kg), c) Diclofenac (100 mg/kg), d) Mexiletine (30 mg/kg). Bar charts show percentage of rats displaying an SFL duration of >10 s per 3 min. (Fisher's exact test revealed ***$p<0.001$ for gabapentin and diclofenac at 90 and 180 min compared to pre-drug values). Scatter plots show individual SFL scores (one-way repeated measures ANOVA followed by Dunnett's multiple comparison test revealed *$p<0.05$ for gabapentin at 180 min compared with pre-drug values, n=8 for all experiments).

Fig. 1.

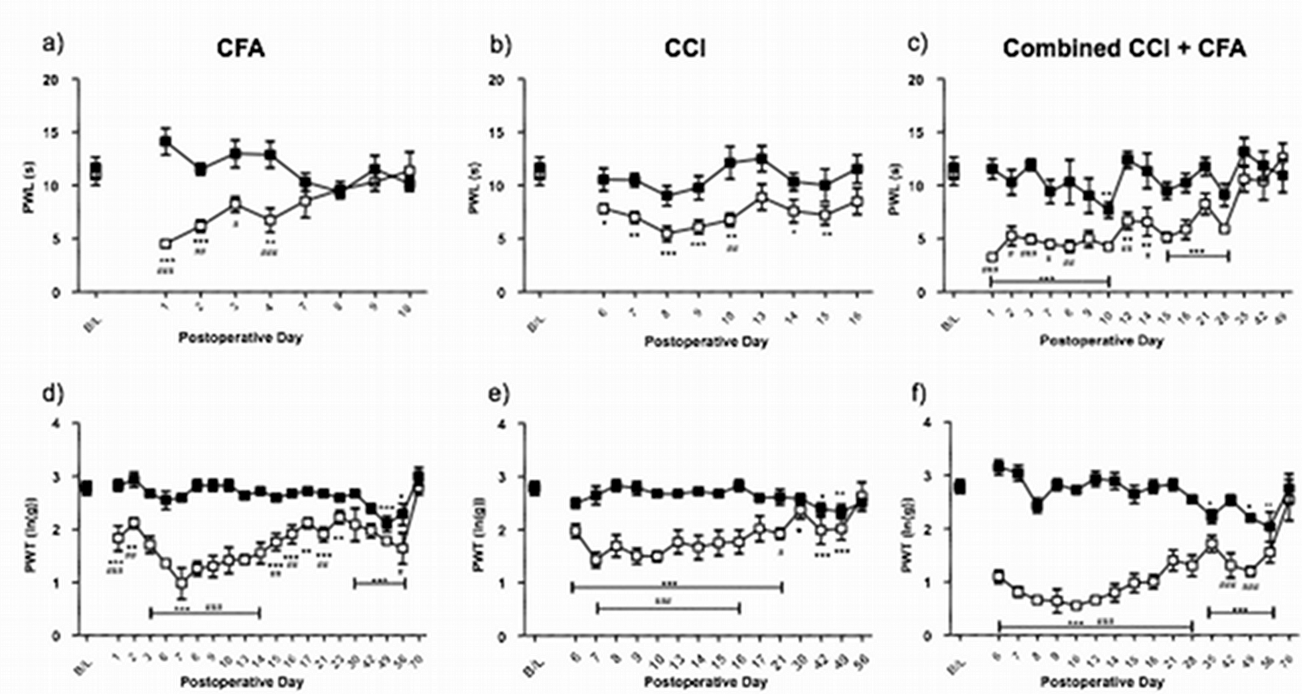

a)
CFA
b)
CCI
c)
Combined CCI + CFA
PWL (s)
Postoperative Day
d)
e)
f)
PWT (mN)
BL

Fig. 2.

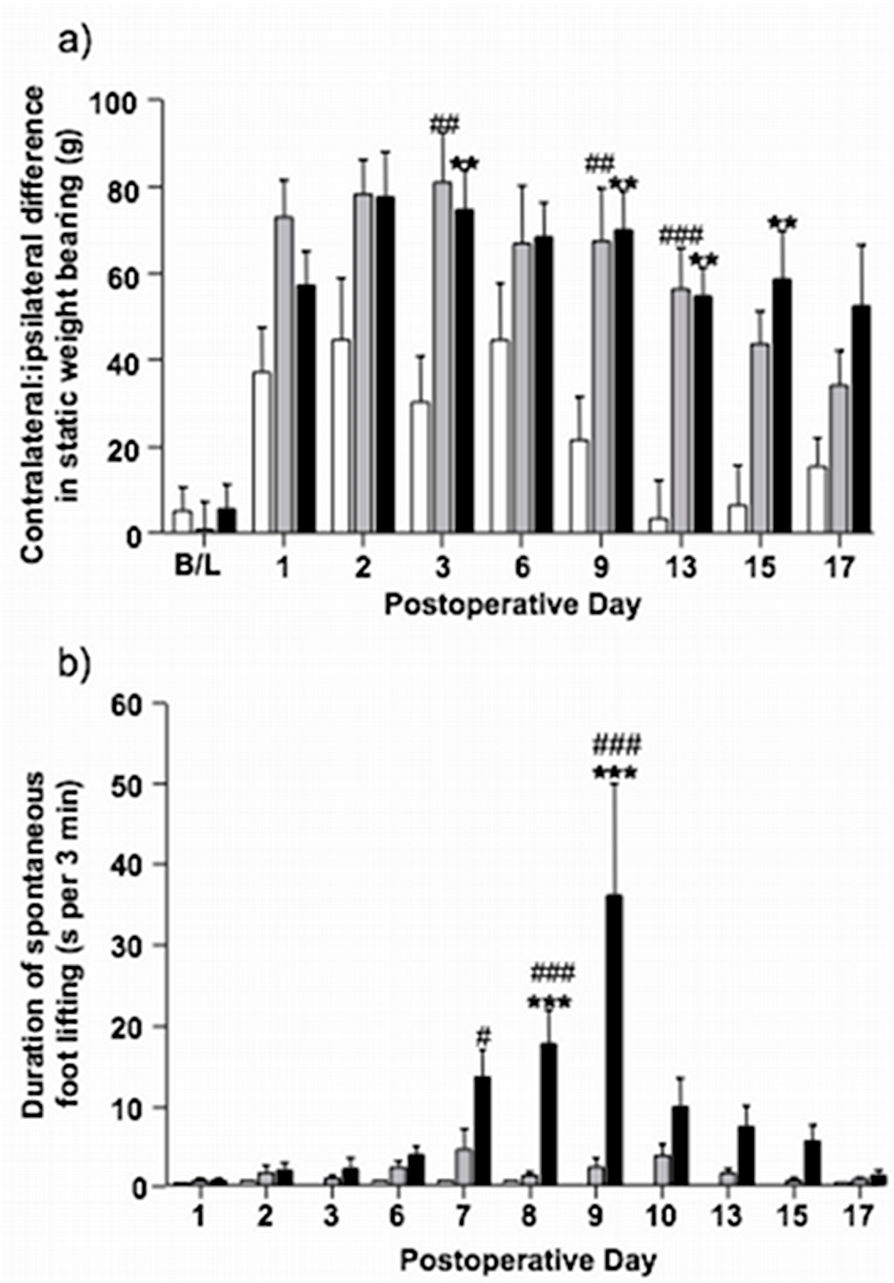

a)
Contralateral:ipsilateral difference in static weight bearing (g)
100
80
60
40
20
0
##
**
##
**
###
**
**
B/L
1
2
3
6
9
13
15
17
Postoperative Day
b)
Duration of spontaneous foot lifting (s per 3 min)
60
50
40
30
20
10
0
#
###
***
###
***
1
2
3
6
7
8
9
10
13
15
17
Postoperative Day

Fig. 3.

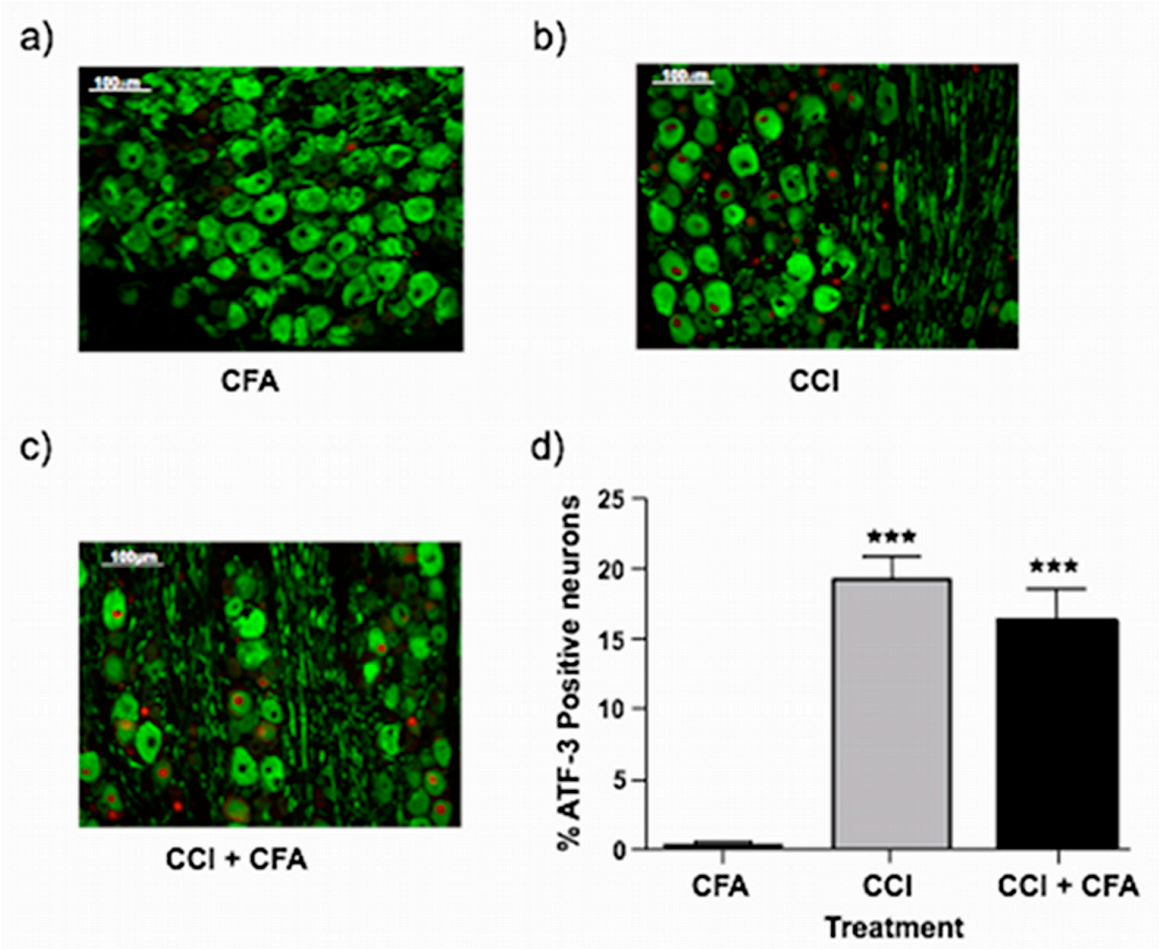

a)
CFA
b)
CCI
c)
CCI + CFA
d)
% ATF-3 Positive neurons
25
20
15
10
5
0
***
***
CFA
CCI
CCI + CFA
Treatment

Fig. 4.

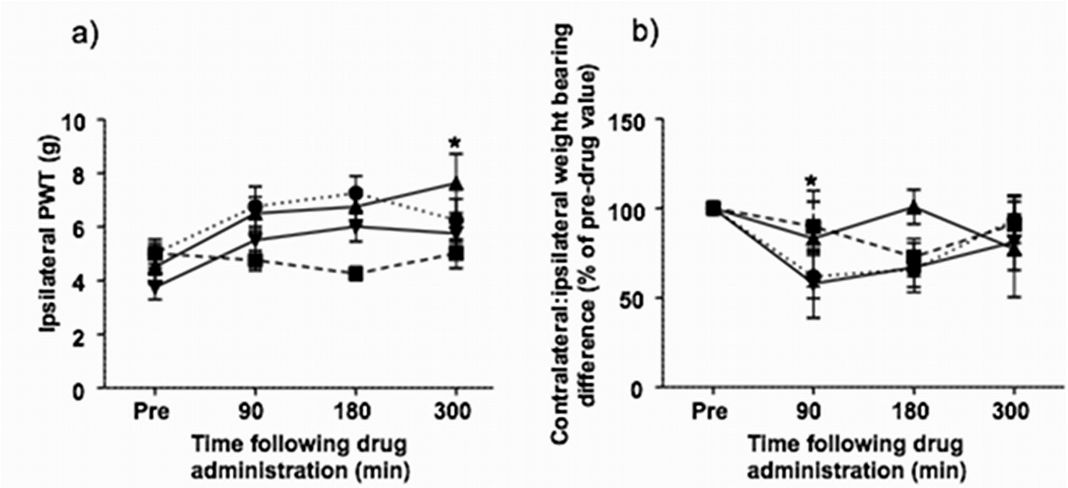
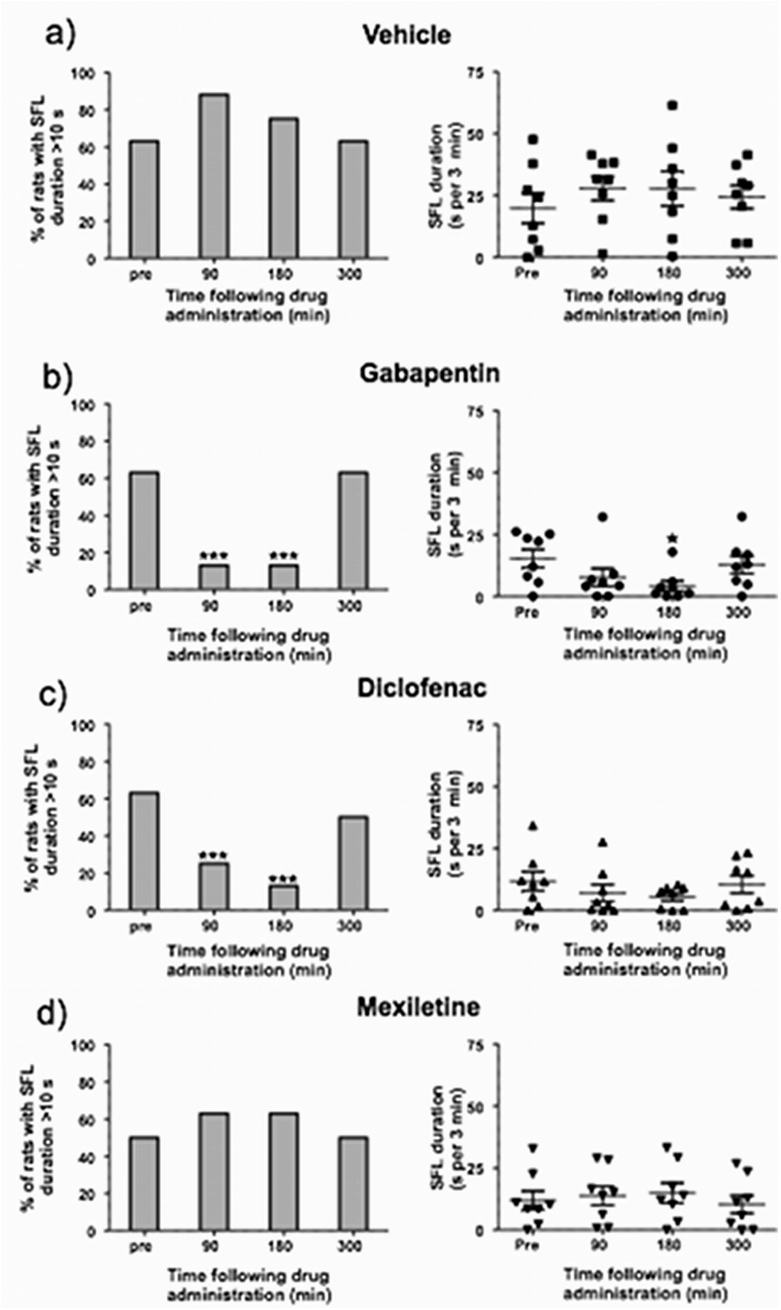

a)
Ipsilateral PWT (g)
10
8
6
4
2
0
Pre
90
180
300
Time following drug administration (min)
b)
Contralateral:ipsilateral weight bearing difference (% of pre-drug value)
150
100
50
0
Pre
90
180
300
Time following drug administration (min)

Fig. 5.


a)
Vehicle
% of rats with SFL duration >10 s
SFL duration (s per 3 min)
pre
90
180
300
Pre
Time following drug administration (min)
b)
Gabapentin
***
c)
Diclofenac
d)
Mexiletine